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A novel experiment of rearing burbot larvae in cages

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Summary

Feeding burbot *Lota lota maculosa* larvae were released in pens deployed in two ponds of the Kootenai River, Idaho, USA drainage: one pond each in 2008 through 2010. The objectives were to compare results of survival, growth, and the practicality of pen rearing burbot. In 2008 we stocked two pens at low density with 30 burbot larvae each (20 larvae per m^3) and collected a total of 47 of the 60 after about 70 days, for an estimated average pen survival of 78%. Average length in August 2008 was 48 mm total length. Yellow perch *Perca flavescens* were discovered in the pond. The pond was drained in December of 2008 to eradicate the yellow perch. In 2009 a total of 467 feeding burbot larvae were placed between five pens at three densities of about 18, 39, 95 burbot larvae per m^3. By September, average TL of burbot was 49 mm. Growth appeared to slow for all stocking densities after the fifth week. Average survival for burbot from initial pen stocking to final recovery was 18%. Best survival was at the two lower densities. In 2010 stocking was moved to a second pond and a total of 484 feeding burbot larvae were placed between five pens at the three densities. In 2010 burbot were supplementally fed. Average survival for the low, moderate, and high density pens was 86, 78, and 20%, respectively. Mean TL at collection in 2010 for the low, moderate and high densities were 37, 37, and 40 mm, respectively. Food availability was believed to be a limiting factor to survival because adding zooplankton appeared to improve survival.

Introduction

Burbot *Lota lota* are abundant throughout much of their natural range (Muth and Smith, 1974; Bruesewitz, 1990; Evenson and Hansen, 1991; Edsall et al., 1993), yet many stocks are diminishing or have been extirpated (Maitland and Lyle, 1990, 1996; Keith and Allardi, 1996; Argent et al., 2000; Arndt and Hutchinson, 2000; Stapanian et al., 2010). Rehabilitation efforts or reintroductions have primarily focused on culture techniques which have been primarily experimental but progressive (Jensen et al., 2008a,b; Vught et al., 2008).

In the Kootenai River of Idaho, USA the burbot population has been in decline since the 1970s (Paragamian et al., 2000) and is on the brink of extirpation (Paragamian et al., 2008). The primary reason was due to habitat changes linked to operations of the Libby Dam in Montana, USA (Paragamian, 2000; Paragamian and Wakkinen, 2008). Any expectations that this population can recover within the next decade are unreasonable even with the most suitable habitat changes in winter discharge, winter temperatures, and improved primary production (Paragamian et al., 2008). Thus, it is of crucial importance that remedial measures to improve this stock's abundance begin immediately not ignoring the importance of habitat changes.

An International Burbot Conservation Strategy was written to formulate a plan to recover burbot in the Kootenai River (KVRI Burbot Committee, 2005; Ireland and Perry 2008). One measure to enhance and rehabilitate the Kootenai River burbot stock was through the introduction of progeny of a donor stock. Intensive culture techniques (rearing fish indoors under controlled environmental conditions) for burbot, although progressing, are not standardized sufficiently to reliably provide large numbers of young for stocking (Jensen et al., 2008a; Vught et al., 2008). But extensive rearing of burbot eyed embryos or larvae to fingerling length has been shown effective in burbot restoration in western Europe (Dillen et al., 2008; Vught et al., 2008) and in the interim may also be a valuable tool in burbot rehabilitation in the Kootenai River. Extensive rearing is the process of raising fish in an outside environment where there is less environmental control than in intensive culture. A third method of rearing is in an outside environment but confined to a pen or cage (Collins, 1970; Mitzner and Middendorf, 1975; Newton and Robinson, 1981; Masser, 1988). Under pen rearing conditions fish are protected from piscivores and can be fed a controlled diet. Growth is easily monitored.

This pilot study examines the effects of different stocking densities on growth and survival of feeding burbot larvae under pen rearing conditions. Our goal was to determine the practicality of rehabilitating burbot populations by rearing burbot larvae in pens and subsequently stocking survivors in local waters.

Study area

Private ponds were selected for the placement of pens for experimental extensive rearing. In the first 2 years of study (2008 and 2009) a pond was selected on private property (Frederick's Pond), in the Kootenai River drainage of Boundary County, Idaho. The rectangular pond was 79 × 37 m, about 2.4 m in depth, and 0.29 ha. The pond was isolated in that the water source was seepage from under a county road and a dyke separated it from Burton Creek, has surface runoff, the outlet drains into a pasture, and was fenced from livestock. The pond was reported to be typically ice covered each winter. The pond was surveyed to create a bathymetric map as well as analysis of water chemistry, zooplankton, and benthic macroinvertebrates (Wilhelm, 2010). The pond did not thermally stratify and the entire bottom of the pond was covered with aquatic macrophytes. The pond had a TN:TP ratio indicating P-limitation, however, the trophic state index indicated the pond was eutrophic (Wilhelm, 2010). The pond was also found to be occupied by potential predators yellow perch *Perca flavescens*, and by predacious species of hemipterans and coleopterans. In 2010 an alternative fish free pond was used, Cow Creek pond. This pond was about 1.5 m deep and had a surface area of 0.059 ha.

Methods

Burbot brood source

Adult brood fish were captured in November 2006 and February 2008, 2009, and 2010 from Moyie Lake, British Columbia, Canada using baited cod traps (Spence, 2000; Neufeld and Spence, 2005) and angling. Moyie Lake is in the Kootenai River drainage and located about 20 km north of Idaho. Burbot larvae for this study were provided by the University of Idaho Aquaculture Research Institute (UIARI), Moscow, Idaho (Jensen et al., 2008a,b).

Cages

Two sizes of cages were used for this investigation. Dimensions for the smaller cages were $1.83 \times 0.91 \times 0.91$-m [volume = 1.53 m^3 (1530 L)] and mesh was 500 μm nitex™. The larger cage was $1.83 \times 1.83 \times 0.91$-m [volume = 3.66-$m^3$ (3660 L)]. Four small cages and one large cage were deployed each in 2009 and 2010. A single HOBO® temperature logger was deployed in the pond to monitor daily temperatures from April to October in 2008 and 2010. We did not monitor temperature in 2009.

Larval releases, stocking densities, and sampling

Pen rearing was implemented with three different treatments by releasing a total of 60 larvae (20 individuals per m^3) in two of the small (1.53 m^3) cages at Frederick's pond in 2008. In 2009, 467 larvae were placed in five cages (4 small and 1 large) within the pond. Burbot larvae were stocked at three different rearing densities: approximately 18 and 39 fish per m^3 in two of the small cages each, and 95 fish per m^3 in the large pen. In 2010 five cages (4 small, 1 large) were transferred to Cow Creek Pond. A total of 484 feeding burbot larvae were placed in the five cages within the pond, stocked at the same respective densities as in 2009. Burbot were released into the cages in May of each year and removed in late August or early September to assess total survival and growth. Differences in burbot survival for low, medium, and high densities within years, between years, between treatments, and differences in non-additional feeding and additional feeding were calculated with the Fischer exact test (Preacher, 2001).

Light trapping

Light traps (Fisher et al., 1996) were used to assess burbot. One light trap in each of the two cages was used in 2008 for a total cage light trap effort of 528 h, in 2009 light traps were set in the cages for a total effort of 1896 h, and in 2010 total light trap effort was 792 h.

Food abundance

In August 2008 to sample zooplankton in Frederick's Pond, triplicate samples using a 0.3-m diameter 80-μm mesh Wisconsin style plankton net was hauled from 1.8 m to the surface. Samples were then washed to the cod end of the net, transferred to pre-labeled vials and preserved with 4% buffered formalin. For analysis, the formalin was removed by washing with water through a 40 μm-mesh screen. The sample was then made to a known volume and a 1–2 ml sub-sample was removed and all zooplankton were counted and identified (Wilhelm, 2010).

In 2010, we provided an additional food supply for the burbot in the Cow Creek Pond cages by collecting zooplankton with a Turtox style plankton net from an offsite location (net was 25.4-cm in diameter and 914.4-cm long). Tows were approximately 6 m in length of filtered pond water. Samples were transferred to the Cow Creek Pond cages and released on June 22, July 8, 15 and 22nd. On average we added about 121, 92, 252 and 267 zooplankton on each day, respectively, (or 78, 59, 162, and 171 zooplankton per m^3 for most cages) comprised of cladocerans, Acroperus, Copepoda and rotifers. The most abundant zooplanktors were Daphnia, Alonella, and Cyclopoids.

Statistical analysis

The Fisher exact test was used to compare treatments within years, between years, between treatments, and between non-fed and fed treatments (Preacher, 2001).

Results

Cage rearing 2008

A total of 84 larvae were captured in the light traps. Daily growth for all cages ranged from 0.00 to 4.28 mm per day with an average daily growth of 0.82 mm per day. Weekly growth of larvae averaged 6.49 mm per week (Figs 1, 2, and 3). Burbot in cages were inventoried and removed in late August 2008. We collected 47 of the 60 burbot larva released in May for a cage survival of 78% (Table 1) and average length was 48 mm TL (range: 31–55 mm). All burbot appeared healthy. When viewed under a microscope the digestive tracts were full of zooplankton.

Cage rearing 2009

A total of 223 burbot larvae were captured in the light traps. Daily growth for all cages ranged from 0.57 to 1.58 mm per day with an average daily growth of 0.35 mm per day. Weekly growth of larvae ranged from a low of 3.98 mm to a high of 11.05 mm and averaging 2.45 mm per week (Figs 2 and 3). By September average TL was 49 mm, ranging from 39 to 58 mm. Most fish were too small (< 1 g) to weigh accurately. Growth overall appeared to slow for all stocking densities after the fifth week.

We removed burbot from all cages on September 2, 2009. Average survival for burbot from initial cage stocking to final recovery was 18% ranging from 6.6 to 60% (Table 2). After measurements all burbot were transported to the Kootenai Tribe of Idaho Hatchery (KTOI) for holding, prior to release into the Kootenai River or a tributary.

Cage rearing 2010

Daily growth for all burbot in cages ranged from 0.68 to 1.38 mm per day with an average daily growth of 0.87 mm per

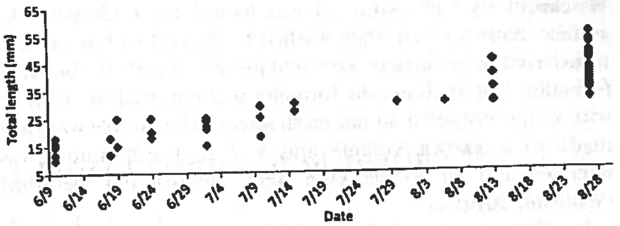

Fig. 1. Larval burbot length at capture in net pens in 2008, captures with light traps

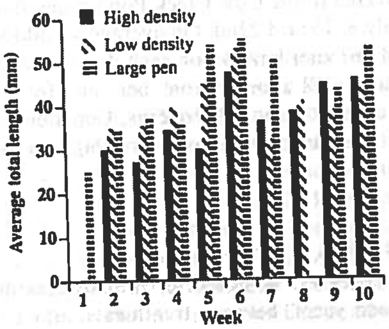

Fig. 2. Average weekly growth of burbot at low, medium and high density in pens at Frederick's Pond over a 10 week period for 2008, 2009, and 2010

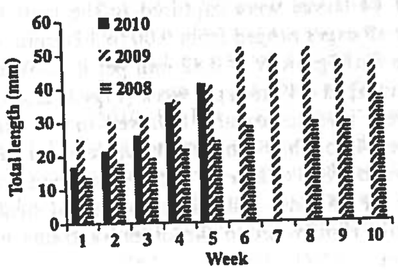

Fig. 3. Average weekly combined growth of burbot in pens for 2008, 2009, and 2010

Table 1
Cage number, volume, stocking numbers (N), density (N m^{-3}), and survival (%) in Frederick's Pond 2008

Cage	Volume (m^3)	Starting fish N	Original stocking density (fish per m^3)	Ending fish N	Ending density (fish per m^3)	Survival (%)
1	1.53	30	19.6	27	17.6	90.0
2	1.53	30	19.6	20	13.1	66.7
Total		60		47		78.4

day. Weekly growth of larvae ranged from a low of 4.49 mm to a high of 9.67 mm averaging 6.12 mm per week (Figs 2 and 3). On July 29, 2010 burbot were collected from the cages and tagged with a VIE tag prior to release. Average survival for the low, moderate, and high density cages were 86, 78, and 20%, respectively (Table 3). Length at collection, in all net cages, ranged from 25 to 70 mm TL while the means for the low, moderate, and high densities were 37, 37, and 40 mm TL, respectively.

Table 2
Cage number, volume (m^3), stocking numbers, density (m^3), and survival in Frederick's Pond 2009

Cage	Volume (m^3)	Starting fish N	Original stocking density (fish per m^3)	Ending fish N	Ending density (fish per m^3)	Survival (%)
1	1.53	27	17.6	10	6.5	37.0
2	1.53	30	19.6	15	9.8	50.0
3	1.53	60	39.2	36	39.2	60.0
4	1.53	60	39.2	16	39.2	26.7
5	3.06	290	94.8	19	6.2	6.6
Total		467		96		

Table 3
Pen number, volume, stocking numbers, density, and survival in Frederick's Pond 2010

Cage	Volume (m^3)	Starting fish N	Original stocking density (fish per m^3)	Ending fish N	Ending density (fish per m^3)	Survival (%)
1	1.53	30	19.6	29	19.0	96.7
2	1.53	30	19.6	23	14.4	76.7
3	1.53	60	39.2	48	31.4	80.0
4	1.53	60	39.2	46	30.1	76.7
5	3.06	304	99.3	60	19.6	19.7
Total		484		154		31.4

Food abundance

In August 2008 the zooplankton community in Frederick's Pond was comprised of small species of cladocerans, possibly indicating size-selective predation by predators (Wilhelm, 2010), cladocerans; *Bosmina* sp. were most dense at 82 100 individuals per m^3 (SE = 1300.0), with *Ceriodaphnia* sp. second at 56 500 m^{-3} (SE = 3700) and *Diaphanasoma* sp. third at 100 m^{-3} (SE = 100). Copepod adults were relatively scarce. In contrast, copepod nauplii were dominant at 111 900 m^{-3} (SE = 8900), with calanoids at 1610 m^{-3} (SE = 2500), and cyclopoids at 5500 m^{-3} (SE = 1200).

On May 7, 2010 a zooplankton survey was conducted at Cow Creek Pond before deployment of cages. Zooplankton densities were; *Daphnia* most abundant at 34 900 m^{-3} (SE = 5800), with *Ceriodaphnia* second at 10 000 m^{-3} (SE < 1000) and *Diaphanasoma* last at < 1000 m^{-3} (SE < 1000), while copepods were relatively scarce, copepod nauplii dominated at 9832 m^{-3} (SE = 3300), Cyclopoids at 34 900 m^{-3} (SE = 150), and Calanoids at < 1000 m^{-3} (SE < 1000).

Statistical analysis

The Fisher exact test, used to compare within treatments by year, indicated there was no difference in low density cages for 2008, 2009, and 2010 (P = 0.56, P = 0.63, and P = 0.57, respectively). Between the years 2008 and 2009 there was no difference (P = 0.07), but a difference of higher survival was found for 2010 vs 2009 (P = 0.027), but none between 2008 and 2010 (P = 0.786).

ledium density comparisons within years (2009 and 2010) wed a difference between cages for 2009 ($P = 0.029$) but no erence between cages in 2010 ($P = 0.89$) while a difference found at medium densities between 2009 and 2010 $= 0.006$). Dropping the low survival cage in 2009 (Table 2) ilted in no difference between years ($P = 0.82$). A signif- it difference in survival for the high density cages, one each 009 and 2010, was detected ($P < 0.001$).

eeding burbot additional zooplankton in 2010 did not rove survival in low density cages compared to previous rs ($P = 0.183$) but appeared to improve survival in lium, and high density cages compared to each treatment years they were not fed ($P = 0.006$ and $P < 0.001$, ectively).

lerick's and Cow Creek pond mean temperatures

!008 Frederick's Pond mean temperatures ranged from 5.4 9.7°C with an average of 13.9°C. We did not monitor Cow ek pond in 2008. In 2009, we did not monitor either derick's Pond or Cow Creek Pond with a temperature order. In 2010, Frederick's Pond mean temperatures ranged n 14.3 to 20.3°C with an average of 18.1°C while Cow ek Pond's 2010 temperatures ranged from 9.4 to 15.1°C ı an average of 11.7°C during the study.

cussion

ensive rearing of larval burbot in cages provided a ndation to examine the questions 'is rearing burbot larvae er cage conditions a practical technique and what is the ct of density on survival'. For example the survival rate in es at low densities (20–39 burbot larvae per m^3) without plemental feeding, averaged about 50% over about a ionth period in 2008 and 2009. With supplemental feeding !010, survival was about 86 and 78% at the same densities. there were significant differences within treatments ween years. Survival at higher densities (90 burbot larvae m^3) in cages in 2009 and 2010 was about 6 and 20%, ectively.

lthough this study was not designed to compare cage ring directly to intensive rearing, survival was higher than lings of other studies using a variety of intensive culture iniques. Under experimental intensive culture conditions, l feeding *Brachionus calyciflorus* and *Aretemia*, Vught et al. 08) had 4% survival with 5000 larvae per m^3 (Harzevilli ıl., 2003). Jensen et al. (2008b) experimented with incuba- ı jars and feeding of various zooplankton and commercial d diets had a survival range of 0 to 9.2% in year 1 and 0.4 9.2% survival in year two with stocking densities of 2500 l 25 000 larvae per m^3, respectively. In extensive rearing dies at Cow Creek Pond in 2009, burbot larvae had a vival rate of 0.65% (30 survivors of 4600 fish stocked at ge for roughly 120 days; James Barron, University of Idaho .I [UIARI], pers. comm.). In a semi-intensive culture in '8 UIARI survival was 0.22 (22 survivors of 10 000 fish; at ge for 109 days). In another semi-intensive culture experi- nt, 194 of 11 025 fish survived at 1.76% (at large for days). In an extensive rearing study, survival of burbot cked directly into Frederick's Pond was found to be about 1% for the same time frame (Paragamian and Laude, 2010) the pond was also found to be occupied by yellow perch. ·bot culture studies have shown larvae have a high level of endence on live food early in life (Harzevilli et al., 2003) and use of commercial diets before they have been effectively weaned; usually have low success (Jensen et al., 2008b).

Feeding burbot larvae in cages in 2010 appeared to improve survival in medium and high density cages over 2009 but did not appear to make a statistical difference for the low density cages, albeit a low sample size in this study. Although adding zooplankton in the high density cage in 2010 improved survival it did not improve it to the higher levels of the low and medium density cages. Further, because zooplankton densities in Cow Creek Pond was less, 102 000 m^{-3} compared to 257 210 m^{-3} in Frederick's Pond, feeding burbot additional zooplankton improved survival in Cow Creel Pond cages. Yet in August 2008 the zooplankton community in Frederick's Pond was comprised of small species of cladocerans, possibly indicating size-selective predation by predators like yellow perch (Wilhelm, 2010). For comparison other waters, zoo- plankton densities in both ponds were in the range of zooplankton density in water bodies with native burbot stocks, 6800–400 000 m^{-3} (Hardy et al., 2008).

Growth of burbot larvae reared in cages at lower densities, feeding at an ambient zooplankton density, grew better than larvae fed under intensive conditions in controlled experiments (Harzevilli et al., 2003; Harzevilli et al., 2004; Jensen et al., 2008b; Vught et al., 2008). However, an unbiased assessment of differences in growth between this study and others was not possible because of differences in experimental design and objectives. But as a point of reference after about 70 days growth of burbot larvae in cages grew from about 9 mm TL at release to an average length in August 2008 of 48 mm TL (range: 31 55 mm) and in 2009 by the first of September averaged 49 mm TL (range: 39–58 mm). In an experiment using different diets, Harzevilli et al. (2003) found differences in growth of burbot larvae from about 5 to 9 mm TL in 35 days. Harzevilli et al. (2004) used different light and temperature conditions and found that the most rapid growth after 20 days occurred in 16°C water, with burbot growing from about 4 to 8 mm TL. Light had little influence on growth rate. However, highest survival occurred at 12°C. In other experiments, burbot larvae feeding on live prey only averaged 13.7 mm TL after 52 days post-hatch (Jensen et al., 2008b). Jensen et al. (2008b) also found that burbot larvae (31 days post-hatch) fed brine shrimp (*Artemia*) only grew better than larvae fed a combination of brine shrimp and a commercial diet (26 and 15.6 mm TL, respectively).

Availability of food was also the most likely limiting factor to burbot growth in cages. In 2008 and 2009 food was not added to the cages and growth was less than in 2010 when zooplankton prey were added. Zooplankton density in 2009 was visibly lower in cages after the fifth week for the two higher densities; growth in 2009 did not slow for the low density trial until the eighth week, and did not slow at all for the low density trial in 2008. It is likely that cannibalism among burbot larvae occurred in 2009 and was in part responsible for the lower survival. After harvest of burbot from cages in 2009 were transferred to circular tanks at the KTOI hatchery. The burbot were checked daily and although fed zooplankton their numbers gradually diminished. While being removed from the tank for release in tributaries, four dead burbot were found that appeared have been injured by conspecifics, one of which appeared to be partially digested. In 2009 most burbot looked thin, while one was about 1.5 times longer than the remainder (the cannibalistic one?). Cannibal- ism in intensive culture at the UIARI was not uncommon (N. Jensen, UIARI, pers. comm.). In 2010 one larvae perished trying to swallow a second smaller larvae but the smaller went out the larger burbot's gill cover rather down its esophagus. As a result burbot were removed sooner from the cages in 2010 than other years.

The temperature profile of Frederick's pond approached 20°C during the summer of 2008. Temperatures of 20°C and more are approaching the upper limit for juvenile burbot rearing in some studies. Taylor and McPhail (2000) believed fluctuations in temperatures during early life of burbot may have a large effect on recruitment. Thus, in summers with exceptionally high air temperatures burbot rearing may be limited to a shorter period.

Cages worked well for providing high early survival of burbot but after about 6 weeks burbot should be released in an environment with much lower competition. Burbot were also easier to harvest from cages than open ponds. Food availability was believed to be limiting factor to growth and survival and future research should experiment further with supplemental feeding of live and commercial food. The practicality of cage rearing burbot cannot be fully addressed by this study alone but a limitation is that without supplemental feeding only low numbers of burbot could be reared in cages of 30–40 m^{-3}. Far more cages than the five tested in this study would be required to rear sufficient numbers of burbot needed for burbot rehabilitation in the Kootenai River of 110 000–900 000 annually (Paragamian and Hansen, 2011).

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Conflict of interest

The authors of this manuscript had no conflicts of interest in regards to the findings or content.

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